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Targeted Adenoviral Vectors

PRINCIPAL INVESTIGATOR: Buck E. Rogers, Ph.D.

CONTRACTING ORGANIZATION: Washington University
Saint Louis, Missouri 63110

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13. ABSTRACT (Maximum 200 Words) An adenovirus encoding the genes for human somatostatin receptor subtype 2 and bacterial cytosine deaminase (AdSTR2CD) was constructed. The SSTR2 allows for non-invasive imaging of gene transfer and therapy with radiolabeled somatostatin analogues. The CD converts the prodrug 5-fluorocytosine (5-FC) to the toxic and radiosensitizing 5-fluorouracil (5-FU). Thus, it is hypothesized that AdSSTR2CD can be used for the simultaneous expression of SSTR2 and CD for the detection and treatment of prostate cancer. The purpose of this report is to state that there was no progress in the previous year due to the PI changing institutions. The PI left his original institution (The University of Alabama at Birmingham) in August 2002. The funds to conduct the proposed research did not transfer to his new institution (Washington University in St. Louis) until July of 2003. It is anticipated that significant progress will be presented for the 2004 annual report and the 2005 final report.				
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Introduction

It is estimated that approximately 37,000 U.S. men died in 1999 from prostate cancer. It is clear that novel treatments for prostate cancer are necessary. Radiolabeled monoclonal antibodies have been used to treat hormone-refractory prostate cancer with limited success. Reasons for these limitations include, bone marrow toxicity from the long serum half-life of the radiolabeled antibody, heterogeneous tumor distribution of the large molecular weight antibody, and low tumor antigen/receptor expression. A strategy to overcome these limitations is to combine peptide radiotherapy with gene therapy. Radiolabeled peptides can overcome problems associated with bone marrow toxicity and tumor penetration due to their small molecular weight, while gene therapy can be used to increase the tumor antigen/receptor expression. Previous studies have shown that an adenovirus encoding for the somatostatin receptor subtype 2 (AdSSTR2) can be used to increase tumor localization of radiolabeled octreotide analogues.

Objective/Hypothesis. The objective of this proposal is to determine if induction of SSTR2 with AdSSTR2 on human prostate cancer xenografts in mice has a therapeutic effect after targeting with the octreotide analogue, ^{64}Cu -octreotide. **Specific Aims.** SPECIFIC AIM #1. Evaluate the expression of SSTR2 on human prostate cancer cells *in vitro* after infection with AdSSTR2 using radiolabeled octreotide binding and internalization assays. SPECIFIC AIM #2. Evaluate the distribution of radiolabeled octreotide after i.v. injection by non-invasive PET imaging and by gamma counter analysis in nude mice bearing s.c. human prostate cancer xenografts injected with AdSSTR2. SPECIFIC AIM #3. Perform therapy studies in a mouse model of human prostate cancer utilizing the best vector as determined in Specific Aims 1 and 2 and ^{64}Cu -octreotide. **Study Design.** The first aim of the study will evaluate SSTR2 expression on PC-3 and DU-145 human prostate cancer cells *in vitro* after infection with AdSSTR2. These assays will be conducted using ^{64}Cu -octreotide in Scatchard and internalization experiments. The Scatchard analysis will determine the level of SSTR2 expression on the cells and the internalization of SSTR2 is important for the subsequent localization and therapy studies. The PC-3 and DU-145 cells will then be implanted s.c. in athymic nude mice and SSTR2 expression will be determined by ^{64}Cu -octreotide tumor localization following injection of AdSSTR2. These studies will be conducted using PET imaging, tissue counting in a gamma counter and immunohistochemistry. Therapy will be conducted in mice bearing subcutaneous PC-3 and DU-145 tumors following injection of AdSSTR2 and i.v. injections of ^{64}Cu -octreotide. **Relevance.**

These studies are directly relevant to improving the treatment of hormone-refractory prostate cancer. Novel therapies are needed for the treatment of this disease and this proposal introduces a new paradigm for its treatment by combining targeted radiolabeled peptide therapy with gene therapy. In addition, this strategy can be used to detect prostate cancer using external PET imaging.

Body

Statement of Work

SPECIFIC AIM #1. Evaluate the expression of SSTR2 on human prostate cancer cells *in vitro* after infection with AdSSTR2 using radiolabeled octreotide binding and internalization assays.

Task1: Months 1-4: Radiolabel octreotide with ^{64}Cu and use the ^{64}Cu -octreotide to determine the level of SSTR2 expression in PC-3 and DU-145 human prostate cancer cells after infection with various amounts of AdSSTR2. This will be done by Scatchard analysis. In addition, internalization of SSTR2 and ^{64}Cu -octreotide will be evaluated.

SPECIFIC AIM #2. Evaluate the distribution of radiolabeled octreotide after i.v. injection by non-invasive PET imaging and by gamma counter analysis in nude mice bearing s.c. human prostate cancer xenografts injected and with AdSSTR2.

Task 1: Months 3-12: Athymic nude mice will be implanted s.c. with PC-3 and DU-145 human prostate cancer cells and injected intratumorally with AdSSTR2 3-5 weeks later. ^{64}Cu -octreotide will then be administered i.v. and the mice imaged using a gamma camera to determine SSTR2 expression and ^{64}Cu -octreotide distribution.

Task 2: Months 3-12: Athymic nude mice will be implanted s.c. with PC-3 and DU-145 human prostate cancer cells and injected intratumorally with AdSSTR2 3-5 weeks later. ^{64}Cu -octreotide will then be administered i.v. and the mice will be sacrificed to determine SSTR2 expression by immunohistochemistry and counting tissues in a gamma counter. These studies will be complementary to those discussed in Task 1.

Task 3: Months 6-15: Athymic nude mice will be implanted s.c. with PC-3 and DU-145 human prostate cancer cells and injected i.v. with AdSSTR2 3-5 weeks later. ^{64}Cu -octreotide will then be administered i.v. and the mice will be sacrificed to determine SSTR2 expression by immunohistochemistry and counting tissues in a gamma counter. These studies will be an initial step towards evaluating this system in the context of hormone-refractory disease. Administration of the vectors i.v. will not be used in therapy studies unless the tumor expression of SSTR2 is at

least two-fold greater than expression in the liver.

SPECIFIC AIM #3. Perform therapy studies in a mouse model of human prostate cancer utilizing the best vector as determined in Specific Aims 1 and 2 and ^{64}Cu -octreotide.

Task 1: Months 13-24: Athymic nude mice will be implanted s.c. with PC-3 and DU-145 human prostate cancer cells and injected intratumorally with AdSSTr2 3-5 weeks later. Various therapeutic doses of ^{64}Cu -octreotide will be administered i.v. 2 and 4 days after adenovirus. Tumors will be measured every 3 days to determine if there is any response to treatment. Controls will include an irrelevant adenovirus injection, unlabeled octreotide injections, and no treatment. Toxicity will also be monitored throughout the studies.

Since the previous annual report, the PI has changed institutions. He left The University of Alabama at Birmingham in August of 2002 and started at Washington University in St. Louis in September of 2003. Due to administrative issues, the funding for this proposal was not transferred to Washington University until July 2003. Therefore, there were no funds available to conduct the proposed research and generate data for this annual report. Anticipated studies are discussed below in the conclusions.

Conclusions

Due to the transfer of institutions and the lack of funds to conduct the proposed research, there was no progress in the previous year. Since funding is now in place, we anticipate completion of specific aims # 1 and #2 discussed above. Overall, we anticipate significant progress over the next year of funding in accordance with the Statement of Work.